Supporting Information

Bridging the gap between molecular and elemental mass spectrometry: Higher energy collisional dissociation (HCD) revealing elemental information

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The supporting material presented consists of Figures S1, S2, S3 and S4. Moreover, some additional comments have been added to Figures S2 and S4.

In Figure S2, the isotope pattern matching for confirmation of the Pt-containing peptides located by the elemental reporter ion after high energy HCD fragmentation is shown. Some of the presented profiles match clearly with the expected profile for a given platinated peptide of similar weight. Some other profiles in Figure S2 also correlate with the expected pattern although some of the less intense isotopes are very small or not present (m/z 2875.28). In this sense the elemental reporter ion is a valuable tool to locate Pt-peptides which cannot be detected with enough sensitivity in the full scan to draw accurately the isotope pattern. Therefore, this fragmentation strategy not only simplifies the search of the target species but also improves the results compared with the time consuming and tedious manual search of modified isotope patterns.

The alternatively use of the high energy HCD fragmentation for imaging purposes is demonstrated in Figure S4. After deposition of two droplets containing Ho-MeCAT-IA and Lu-MeCAT-IA labeled standard peptide WWCNDGR, respectively, consecutive MALDI-MS and LA-ICP-MS analyses were performed. From the images presented it can be conclude that the elemental distribution obtained by ICP-MS can be reproduced using the elemental signal of the reporter ion of the molecular MS technique (Figure S4b, c and e). Although the spatial resolution employed in the MALDI experiment is much lower than the LA image, the first one has the advantage of being able to acquire in the same experiment structural information of concrete species for identification purposes (Figure S4d and f).

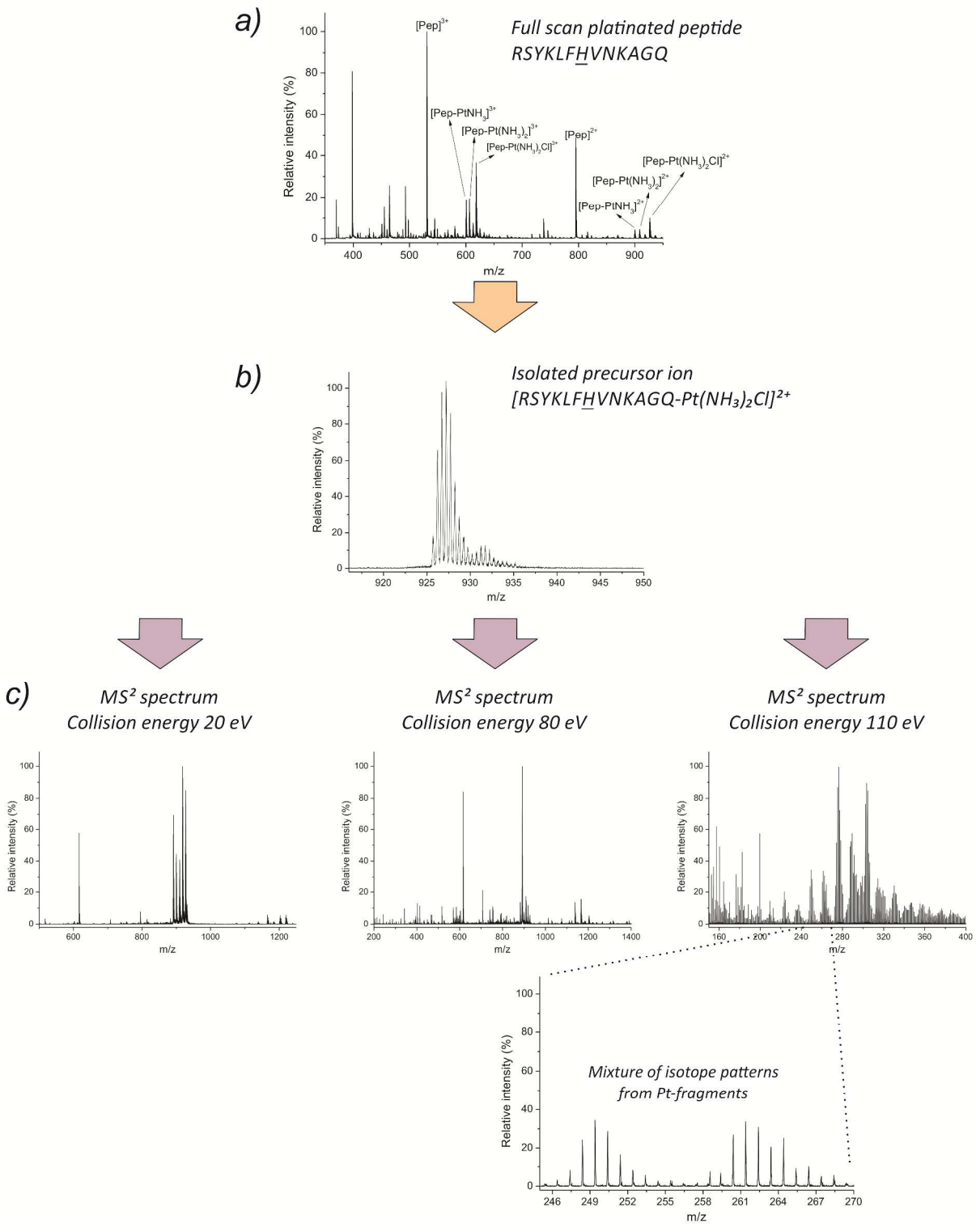

Figure S1. Direct infusion analysis of the platinated peptide RSYKLFHVNKAGQ using an ESI-Q-TOF instrument. a) Full scan, b) isolation of the precursor ion $[RSYKLFHVNKAGQ\text{-}Pt(NH_3)_2Cl]^{2+}$ and c) fragmentation at different collision energies (20, 80 and 110 eV) of the precursor ion.

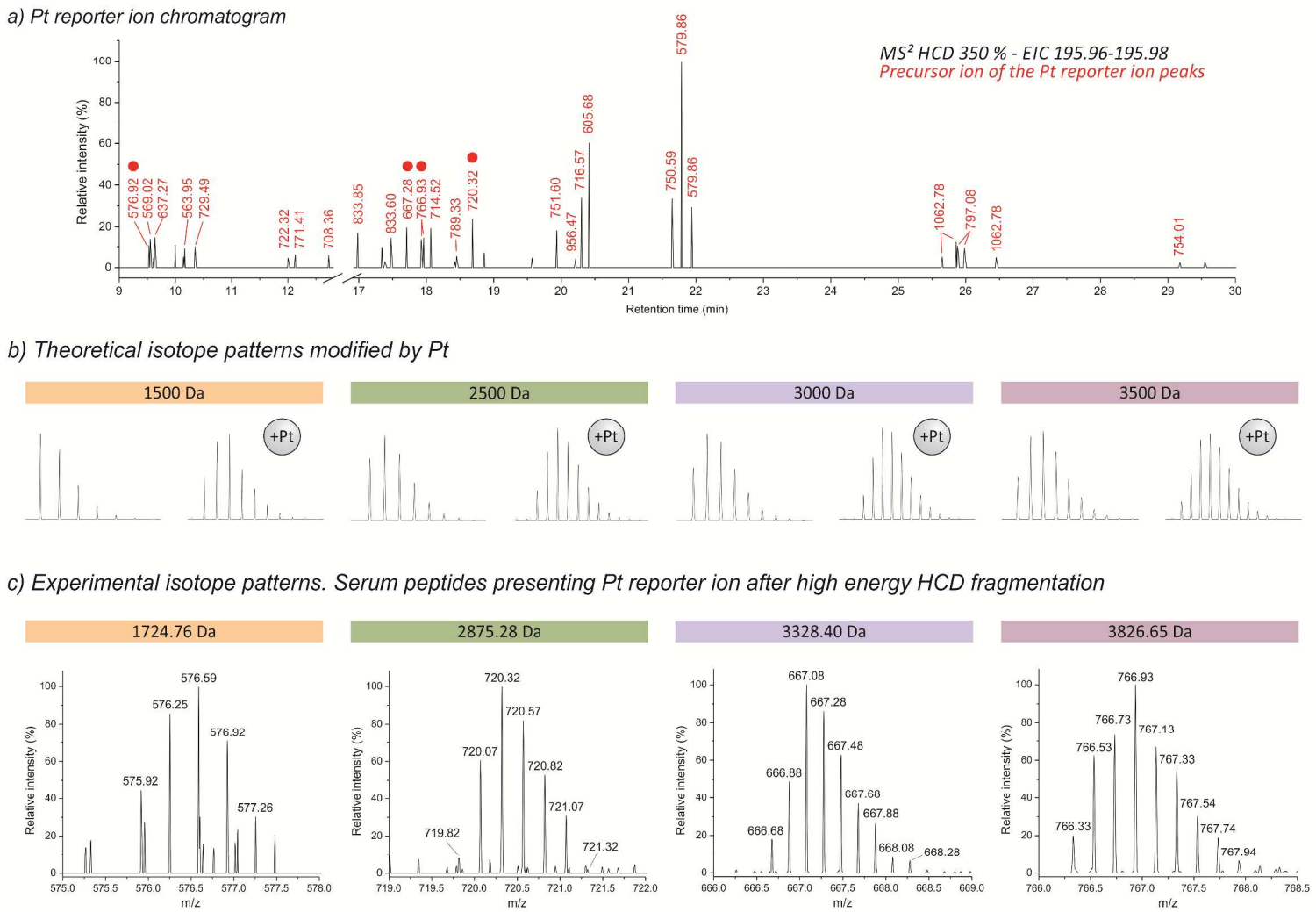

Figure S2. Use of the Pt reporter ion after high energy HCD fragmentation (NCE 350 %) in LC(C18)-ESI-LTQ-Orbitrap analysis for the location of Pt-peptides in a human blood serum sample incubated with cisplatin. a) MS^2 extracted ion chromatogram of the Pt reporter ion isotope at m/z 195.97 (precursor ion from which the reporter ion peak is produced is indicated in red; precursor ions detailed in panel c are indicated with a red dot). b) Comparison of theoretical isotope patterns of Pt-free and Pt-containing peptides at different molecular weights. c) Detail of the acquired isotope pattern of some precursor ions reported as platinated in panel a.

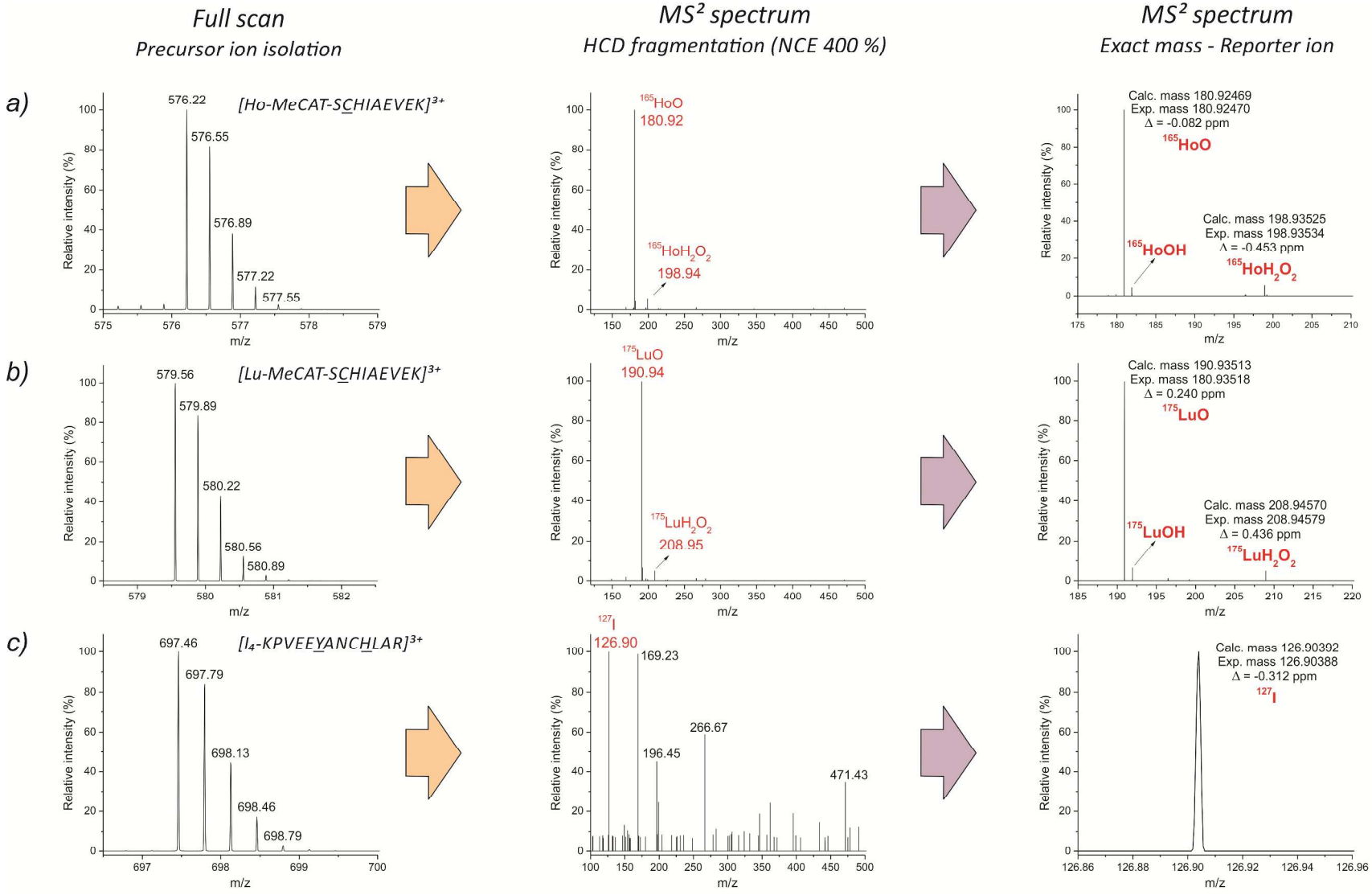

Figure S3. High energy fragmentation (NCE 400%) spectra of peptides containing Ln-MeCAT-IA labels or iodine. a) Ho-MeCAT-SCHIAEVEK, b) Ho-MeCAT-SCHIAEVEK, and c) I_4-KPVEEYANCHLAR. Full scan of the selected precursor ions and MS^2 spectrum are shown. Detailed view of the MS^2 spectrum for identification by exact mass of the elemental reporter ions.

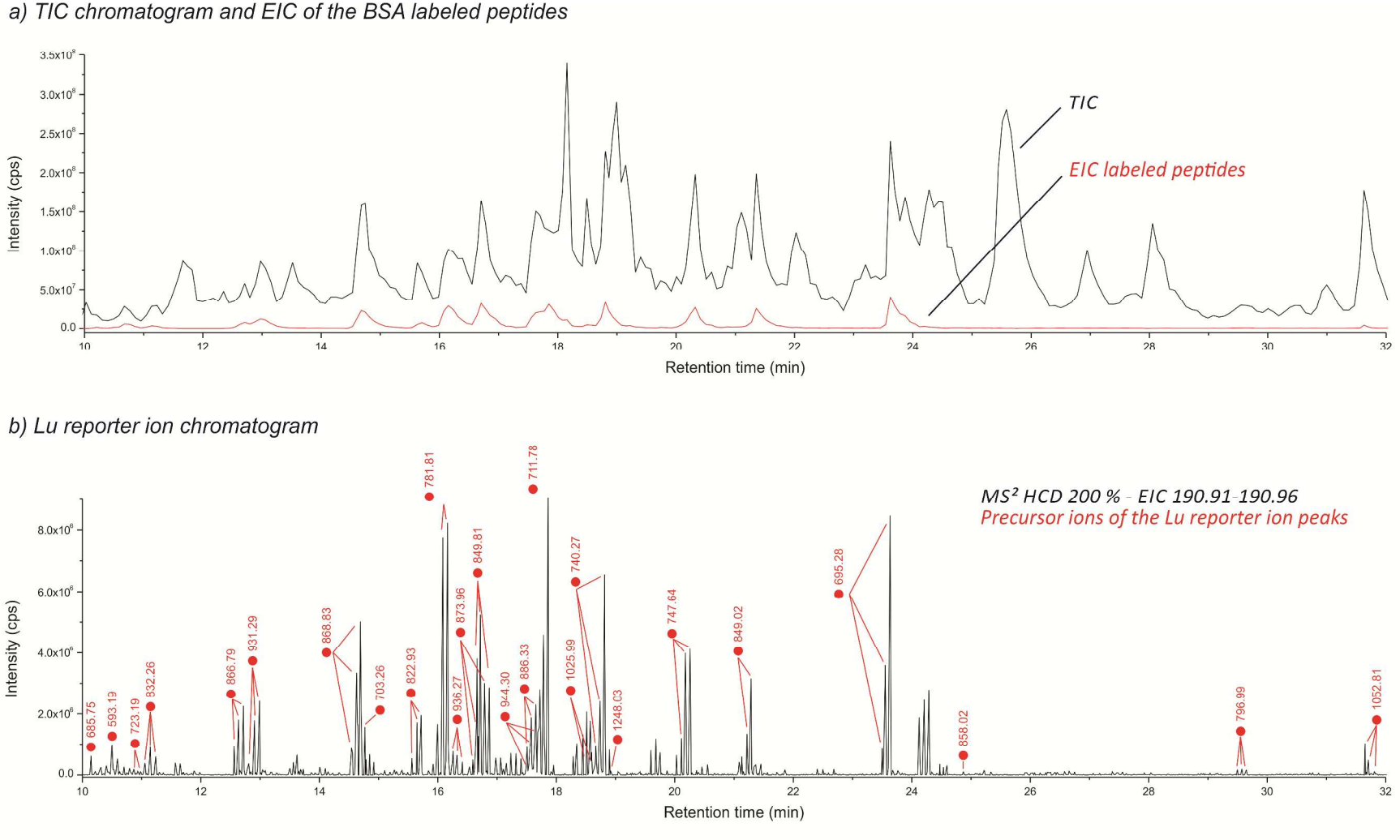

Figure S4. Use of the Lu reporter ion after high energy HCD fragmentation (NCE 200 %) in LC(C18)-ESI-LTQ-Orbitrap analysis for the location of Lu-MeCAT-IA labeled peptides spiked in a human blood serum sample. a) Comparison of the total ion current (TIC) chromatogram (black) and the extracted ion chromatogram (EIC, red) of all the labeled peptides. b) MS^2 EIC of the Lu reporter ion isotope at m/z 190.935 (precursor ion from which the reporter ion peak is produced is indicated in red; precursor ions highlighted in Table S1 are shown with a red dot).

Table S1. List of BSA tryptic peptides, theoretical masses for unlabeled and Lu-MeCAT-IA labeled peptides, expected m/z values for 2+ and 3+ adducts, and experimental elution time in the described chromatographic system.

BSA cysteinyl tryptic peptides	Mass (Da)	Pep-Lu-MeCAT-IA (Da)	m/z $[Pep\text{-}Lu\text{-}MeCAT\text{-}IA]^{2+}$	m/z $[Pep\text{-}Lu\text{-}MeCAT\text{-}IA]^{3+}$	Elution time (min)
DVCK	463.2095	1184.3703	**593.1925**	395.7974	10.49
CASIQK	648.3259	1369.4867	**685.7507**	457.5029	10.67
GACLLPK	700.3936	1421.5544	**711.7845**	474.8588	17.86
LCVLHEK	840.4522	1561.6130	**781.8138**	521.5450	16.15
NECFLSHK	976.4431	1697.6039	**849.8093**	566.8753	16.70
QNCDQFEK	1010.4122	1731.5730	**866.7938**	578.1983	12.71
SHCIAEVEK	1014.4799	1735.6407	**868.8277**	579.5542	14.74
EACFAVEGPK	1049.4846	1770.6454	**886.3300**	591.2224	17.63
SLHTLFGDELCK	1361.6644	2082.8252	1042.4199	**695.2824**	23.62
YICDNQDTISSK	1385.6127	2106.7735	1054.3941	**703.2651**	14.68
CCAADDK	724.2515	2166.5732	1084.2939	**723.1984**	10.86
DDPHACYSTVFDK	1496.6236	2217.7844	1109.8995	**740.2688**	18.80
LKPDPNTLCDEFK	1518.7383	2239.8991	1120.9569	**747.6403**	20.32
MPCTEDYLSLILNR	1666.8053	2387.9661	1194.9904	**796.9960**	29.64
CCTESLVNR	1023.4472	2465.7689	1233.8917	**822.9303**	15.70
CCTKPESER	1051.4421	2493.7638	1247.8892	**832.2619**	11.13
RPCFSALTPDETYVPK	1822.8918	2544.0526	1273.0336	**849.0248**	21.35
LFTFHADICTLPDTEK	1849.8915	2571.0523	1286.5335	**858.0247**	24.84
ECCDKPLLEK	1176.5513	2618.8730	1310.4438	**873.9650**	16.70
TCVADESHAGCEK	1348.5382	2790.8599	1396.4372	**931.2939**	12.98
ETYGDMADCCEK	1363.4725	2805.7942	1403.9044	**936.2720**	16.31
EYEATLEECCAK	1387.5630	2829.8847	1415.9496	**944.3022**	17.63
YNGVFQECCQAEDK	1632.6543	3074.9760	1538.4953	**1025.9993**	18.56
GLVLIAFSQYLQQCPFDEHVK	2434.2350	3155.3958	1578.7052	**1052.8059**	31.63
ECCHGDLLECADDR	1577.5903	3741.0728	1871.5437	**1248.0316**	18.87

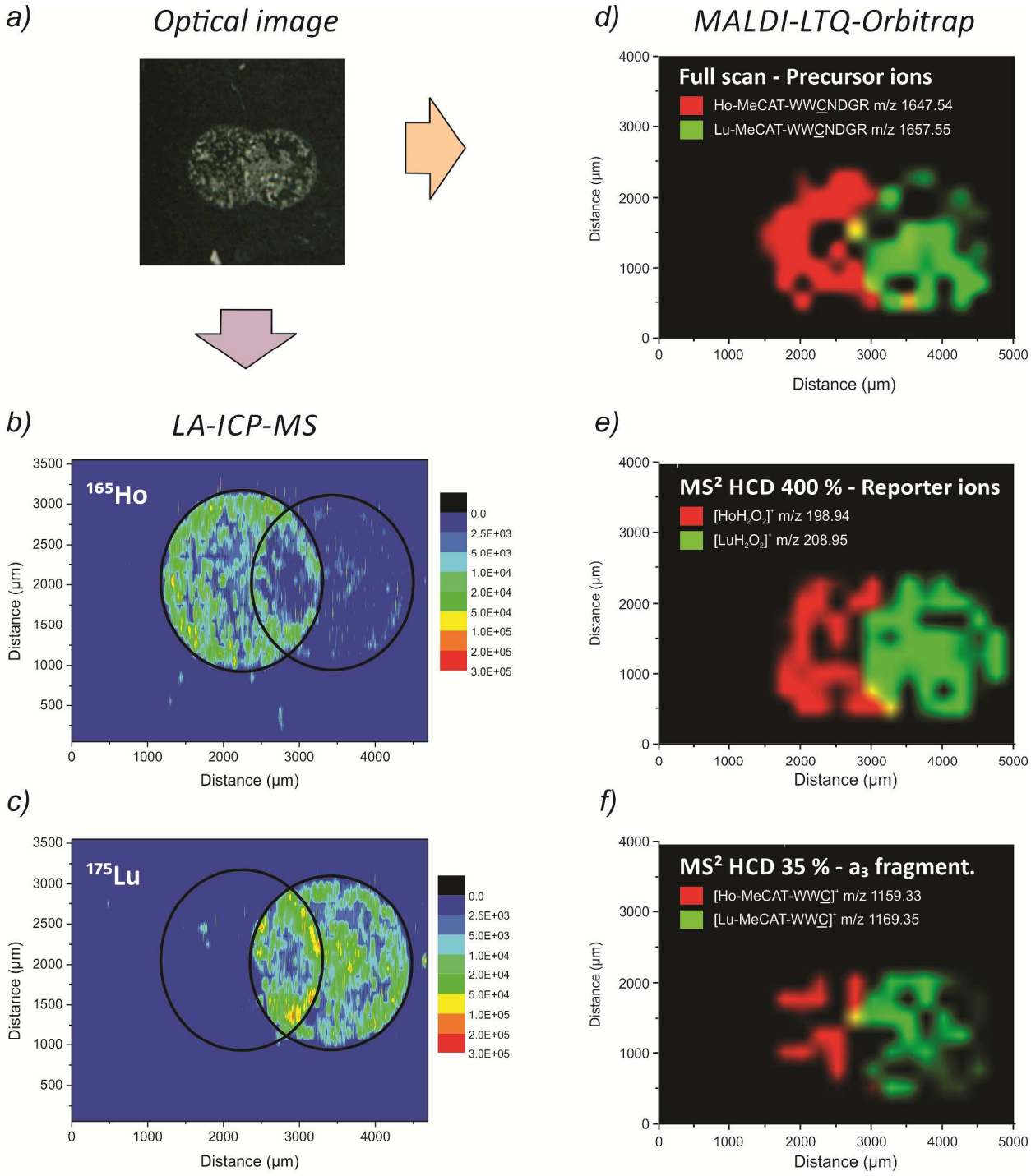

Figure S5. Imaging of two overlapped droplets containing Ho-MeCAT-WWCNDGR and Lu-MeCAT-WWCNDGR. a) Optical image. b) LA-ICP-MS analysis monitoring ^{165}Ho and c) ^{175}Lu. d) MALDI-LTQ-Orbitrap analysis showing the extracted ion image for the intact labeled peptides (precursor ions), e) the elemental reporter ions after HCD fragmentation at NCE 400% and f) the a_3 ion fragment from the peptides after HCD low energy fragmentation (NCE 35 %).